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Detection of Urinary 8-hydroxydeoxyguanosine (8-OHdG) Levels as a Biomarker of Oxidative DNA Damage among Home Industry Workers Exposed to Chromium

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Abstract

Electroplating workers are using chromium during the working process. Clinical and laboratory evidence indicates that exposure of chromium is very toxic if it is inhaled and can lead to oxidative DNA damage. This study was aimed to investigate factors associated to the urinary 8 - OHdG levels as a biomarker of oxidative DNA damage. Sixty six subjects from electroplating home industry in Tegal, Central Java were included. Urinary chromium levels were determined using AAS. The urinary 8-OHdG level as oxidative DNA damage was measured using ELISA. The levels of chromium in all sample were higher than the normal range (median 11.77 µg/ L), the median of urinary 8-OHdG level was 23.83 ng/ml. Eventhough, age and urinary chromium level were not associated with urinary 8-OHdG's levels, there was a significant association between the period of works and the type of jobs to the urinary 8 - OHdG levels.

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Keywords: chromium; 8-OHdG;electroplating worker; period of works; oxidative DNA damage

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1. Introduction

Home industries in Indonesia play an important role since they require a lot of workers. However, those who work at home industries facing the risk of accident and diseases caused by both workers attitude and environment. It is caused by the lack of knowledge and safety standard on occupational health in home industries [1]. Electroplating home industries of cover or plating material for various tools, including house appliances and cars using chromium is important to improve the quality and prevent metal corrosion. Worker engaged in this process are exposed to chromium through inhalation, ingestion and dermal contact. Inhalation is the primary route of occupational exposure to metals [2].

As the heavy metal, Cr (VI) is highly poisonous compared to other Cr forms, and it is potentially dangerous for health [3]. Environmental chromium compounds are commonly used in electroplating, stainles stell production, leather tanning, textile manufacturing and in wood preservation. Its exposured has been shown to have toxic effect, genotoxic, mutagenic and carcinogenic in human and animal [4-7]. Epidemiology study showed that workers who exposed to chromium production and Cr plating have 2-80 times risk to suffer from lung cancer [8]. Cr (VI) exposure in the body mainly through the aerosol inhale can cause health disorder on the respiratory tube, carcinogenic, liver, kidneys, and immune disorder. Some in vitro study indicated that Cr (III) concentration in the cell can cause DNA damage [9]. In human body, Cr (VI) will be reduced by some mechanisms into Cr (III) in the blood and produce reactive oxygen species (ROS). Cr (VI) acute toxicity occur due to strong oxidator that can damage the kidneys, liver, and blood cell through the oxidation reaction [10].

Cr (VI) can easily enter the membrane cell and will be reduced into trivalence shape in the cell [11-12]. Cr (VI) as the strong oxidator can be getting less valence into trivalence shape through Cr (V) and Cr (IV). This process often results free radical that finally activate O_2 and some Reactive Oxigen Species (ROS), ROS produced by these reactions are superoxide (O_2), hydrogen peroxide (H_2O) and hydroxyl radical (-OH). During Cr metabolism, H_2O_2 can be reduced into –OH in Fenton reaction. Oxide is considered to be responsible for DNA damage, H_2O_2 and –OH, and if it is produced in a big amount, it can induced DNA strand breaks and the basic modification related with metal carcinogenecis [13-19]**.** Excess ROS produced in the reduction reaction can lead to an injury of the DNA cell, fat, and protein [20-21]. The excess ROS can also cause lipid peroxidation and oxidative DNA damage [6][13][18] [22]. The reduction of Cr (VI) into Cr (III) generated the shape of reactive intermediate which is together with the oxidative stress and oxidative tissue damage including apostosis modulation p53 gene regulation and it contributes to sitotoxicity, genotoxicity, and carcinogenocity. Cr (VI) exposure can cause various DNA mutation and chromosome damage, and oxidative changes in protein [23].

Kasai et al. stated that the oxidative form of DNA damage can be seen through the asessment of 8-hydroxydeoxyguanosine (8-OHdG) concentration, cellular oxidative stress biomarker during carcinogenesis [24]. Faux et al. study showed that there were production of 8-OHdG in the isolated DNA from the exposure of Cr (VI) and Cr (V). It showed that there was a damage of isolated DNA oxidative with Cr (VI) and Cr (V), an independen mechanism from thiol and the peroxide hydrogen involvement, possibly through Fenton reaction [25]. There are many factors influencing 8-OHdG, like animal species, sex, age, exercise, alcohol, smokes, weight, and nutrition. Therefore, there was an alternative result from human being subject [26]. Level 8-*hydroxydeoxyguanosine* (8-OHdG) in the urine as the oxidative stress indicator can be measured by *enzyme-linked immunesorbent assay* (ELISA) [27], and can be used as an indication of biologically active dosage on the low and middle exposure of Cr (VI) [28]. Cr toxicity in human body was influenced by the dosage and the length of exposure, exposure sustainability, contact mode, age, health status, nutrition status, immune level, sex, and the right tissue exposure to Cr [29]. This study was aimed to investigate factors associated to the urinary 8-OHdG levels as biomarker of oxidative DNA damage.

2. Subject

2. 1. Materials dan Methods

A cross sectional study was conducted to investigate factors related to the urinary 8 - OHdG levels as biomarkers of Oxidative DNA Damage. Sixty six male workers were recruited from 12 chromium plating home industry in Kecamatan Talang, Kabupaten Tegal, Indonesia. Ethical clearance to conduct this study was obtained from Medical and Health Research Ethics Committee, Faculty of Medicine Gadjah Mada University (Ref : KE/FK/993/EC). All subjects were signed a consent form after explanatiuon of an objective of study, procedures, benefit and all the possible risk. All subjects were investigated for information of age, period of works and type of jobs.

A spot urine sample (10 ml) was collected from each subject after 4 hours continuous working. Urine samples were stored in a nitric acid treated polypropylene container at – 20ºC until needed for urinary chromium (5 ml) and 8-OHdG level (5 ml).

Chromium in urine samples was determined using a flameless atomic absorption spectrophotometer (AAS) required with graphite furnace (GF-3000) and auto-sampler (PAL-3000). This method had been recognized as a specific method for direct determination of chromium in human urine and hence is suitable for routine clinical use. Determination of chromium as internal standard added to urine and showed a recovery rate of 98.4 %.

Urinary 8-OHdG level was determined by an ELISA tool according to the manufacturer's instructions (CUSABIO, China). Urine sample was centrifuged at 1500 rpm for 10 minutes before used. Urinary 8-OHdG level were measured using a competitive enzyme linked with immunesorbent assay kit. According to the manufacturer's instructions 50 µL of standards or sample and the horse-radish peroxidase (HRP) conjugated 8-OHdG are added to a microtiter plate well that had been precoated with antibody specific for 8-OHdG and incubated at 37 °C for 1 hour. After the wells were washed three times with wash buffer (200 µl), 50 µl substrate A and 50 µl substrate B was added, and followed by incubation for 15 min at 37 °C. The color reaction was terminated by the addition 50 µl of stop solution. The absorbance of each well was determinated at 450 nm in an Epoch microplate reader. The determination range was 2–800 ng/mL for 8-OHdG. For each experiment, an 8-OHdG standard curve was constructed (2–800 ng/mL) and a curve-fitting software program (Curve Expert 1.3) was used to quantify 8-OHdG in urine samples [27].

2.2.. Statistical Analysis

The median was used to describe the average and variation for quantitative data after ascertaining the normality by Kolmogorov-Smirnov Z test. The differences between 2 groups were assessed using chi square test to compare data of urinary chromium, age, period of work, type of jobs and urinary 8-OHdG level. Multivariate analysis (binnary logistic) was used to analysis factors associated with the urinary 8 – OHdG levels as biomarkers of oxidative DNA damage. A two sided *p* value below 0.05 was considered significant. The result expressed in p (x) which probability of the occurance of urinary 8-OhdG level and type of job with category 1 = risky and 0 = not risky, work period over median (> 14 years) is category 1= risky and work period of < 14 years category 0 = not risky.

3. Result and Discussion

Sixty six electroplating male workers were included in this study. Table 1 presenting the distribution data after ascertaining the normality by by Kolmogorov- Smirnov Z test.

Table.1. Distribution of selected characteristics of participant.

Variabels	N (%) n (%)*	Range	*P*	95 % CI
Age[a]		34.08± 8.916	0.20	31.88-36.27
> 34 years	36 (54.54)			
≤ 34 years	30 (45.45)			
Type of jobs[b]		1.00±0.500	0.00	1.32-1.56
Dye work	37 (56.06)			
Non Dye work	27 (43.94)			
Period of work[b]		14.00±7.871	0.00	11.90 – 15.77
> 14 years	31 (46.97)			
≤ 14 years	35 (53.03)			
Urinary chromium[b]		11.77±28.828	0.00	14.87- 27.08
> 11.77µg/L	32 (48.48)			
< 11.77 µg/L	34 (51.52)			
Level of urinary 8-OHdG[b]		23.83 ± 149.991	0.00	32.50-106.24
> 22.83 ng/mL	33 (50.00)			
< 22.83 ng/mL	33 (50.00)			

* N: number of sample; %: number of sample percentage
[a] data was reported in the form of mean ± SD
[b] data was reported in the form of ± SD

Tablel 1 shows that only age variable was normally distributed ($p = 0.20$) while the type of work variable, period of works of urinary chromium and urinary 8-OHdG level were not normally distributed. All respondents were not using PPE (Personal Protective Equipment). The median of urinary chromium level was 11.77 µg/L (range 2.811µg/L-145.340µg/L). While median urinary 8-OHdG level was 23.83ng/mL (range1.079 ng/mL-974.990 ng/mL). Chromium level in the urine of the workers in this study is scored minimum 2. 811µg/L and maximum 145.340 µg/L. This score is higher compared to normal range for urinary chromium for human being that is between 0.1 µg/L-0.5 µg/L [30]. While the recommendation of international standard of ACGIH-2005 [31]. The occupational Safety and Health Administration (OSHA) has established an 8 hour-time weighted average (TWA) exposure limit of 5 µg of Cr (VI) per cubic meter of air (5 $\mu g/m^3$) [32].The process of electroplating involves: cleaning, plating and post –treatment of articles. Occupational exposure to chromium occurs mainly through inhalation and dermal absorption in the work environment [2][27]. Cr (VI) enters the body mainly through inhalation, moreover through ingestion and dermal contact. After worker exposured to chromium by inhalation urinary concentration of chromium were found to be increased indicating respiratory absorption [33]. According to Miksche and Lewarter, chromium level in the urine, plasma, and organs shows that the body has already been exposured by chromium. The determination of urine chromium was considered as an indicator from chromium exposure [34]. All workers do not use PPE during the working hour. Worker at electroplating home industry received much less training of occupational health and safety. Lack of knowledge would lead to less awareness of PPE during the working hours including wearing masker, long sleeves shirt, and latex gloves to reduce chromium exposure. Therefore, the use of the just right PPE can lower the exposure level [29]. Cr (VI) exposure can be traced by measuring the chromium level in the blood or urine. The chromium level in the blood or urine reflects the current exposure, but not reflects total chronic chromium exposure including Cr (III) and Cr (VI) [35]. Cr III was quickly exerted through urine and less poisonous because of the poor permiability membrane while Cr (VI) compound penetrate membrane and induced the DNA damage and carcinogenesis [36].

Chi square ($X^{2)}$ test was used to see the difference of percentage between 2 data group and to find out the association between 2 tested variables. Age variable was grouped into 2 that is >34 year and ≤ 34 years. Type of jobs variable was grouped into 2 that was dye and non-dye work. Period of works variable was divided into > 14 years and < 14 years. Urinary chromium variable was grouped into > 11.77µg/L and < 11.77µg/L. While the levels

of urinary 8-OhdG variable was grouped into perils (> 22.83 ng/mL) and non perils (< 22.83 ng/mL). Table 2 shows the result analyses using X^2 test among variables.

Tabel 2. Result of X^2 test among age, type of jobs, period of work, urinary chromium with urinary 8-OHdG levels

Variabels	*P*	OR	95 % CI
Age	0.458	1.683	0.616-4.340
Type of jobs	0.047[x]	3.121	1.133- 8.603
Period of work	0.003[x]	5.333	1.859 -15.301
Urinary chromium	0.218	0.479	0.179-1.279

[x]P < 0.05

Chromium toxicity in the body was influenced by the dosage and the length of exposure, the sustainability of exposure, way to contact, age, health status, sex, and type of tissues exposured by the chromium [37]. All workers in this study were male and they were 8 hours exposured during the working hours. The health effects and toxicity of chromium are orimaly related to the oxidation state of the metal of time of exposure [38]. This study shows that period of work had association with urinary 8-OhdG level. The longer period of work has 5.33 times risk higher of urinary 8-OhdG exposure compared to those who have shorter period of works. It is in accordance with WHO which were stated that the period of works is closely related with working effect disease [38]. Although some study stated that there was an association between age exposure and urinary 8-OHdG level, in this study, there was no association between age and urinary 8-OHdG level. Zhang's study on the electroplating in China was also showed that urinary 8-OHdG only had significant association in the controlled group while in the electroplating worker group there was no significant relationship [27].

While the period of works and job type had significant association with the urinary 8-OHdG levels (p<0.05) (Table 2). Recent study stated that chromium exposure can be carcinogenic and genotoxic [29][36]. Cr (VI) can induce formation 8-OHdG as well, one of major oxidative adduct induced by radical damage to DNA [24]. Using urinary 8-OHdG as indicator of oxidative stress in the cell is common since it is non invasive and easy to apply. The result of bivariate test and multivariate test showed that there was a significant correlated between electroplating job type with the level 8-OHdG in urine (p < 0,005) (Table 2). Individual who works at dye work set relatively closed to electroplating sink compared to those who works at non dye work. According to Sarkar, the metal plating worker who were exposed to the particle and Cr (VI) smoke as a result of explosion on the surface of the liquid in the electroplating sink derived from oxigen bubble and hydrogen came out from the electrode during the plating process [39].

Binnary logistic regression analyses was done to assess correlation between variables (type of jobs, period of work and urinary chromium) found that the period of works variable had the most influence toward the urinary 8-OhdG levels with p value 0.001 and OR number 14.69. Value α was – 0.279 while value β was 2.687 with e value was constanta 2.7182818 [40-41]. The result showed p(x) = 0.9174353 (91.74 %) representing the probability for the occurance of risky urinary 8-OhdG levels was 91.74%.

Zhang stated that Cr low exposure can cause DNA damage which was proven by the finding of level 8-OHdG in the urine [27]. Another study showed an ivolvement of the oxidative damage pathway in the mechanism of toxicity of chromium in occupationally exposed individuals [42]. Although all chromium level in the urine in this study was higher compared to those of the normal level in the human body and the probability for the occurance of risky levels of urinary 8-OhdG is 91.74%, there was no significant correlation between urinary chromium and urinary 8-OHdG levels. This result was not corresponding with Kuo study which stated that there was a positive correlation between urinary 8-OHdG concentrations and urinary Cr concentration [43]. There are many factors

which can affect the 8-OhdG level, such as sex, age, exercise, alcohol, smoking, weight, and nutrition. Therefore, there is a higher degree of variation in results obtained from human subjects.

4. Conclusion

This study indicated that there is higher level of urinary chromium exposure and DNA damage is indicated in electroplating workers by measuring the urinary 8- OHdG levels.

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